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Lipoprotein effects of incretin analogs and dipeptidyl peptidase 4 inhibitors

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Abstract

Elevated post-prandial lipoprotein levels are common in patients with type 2 diabetes. Post-prandial lipoprotein alterations in type 2 diabetics are widely believed to drive inflammation and are considered a major risk factor for cardiovascular disease in diabetic patients. The incretins glucagon like peptide-1 (GLP-1) and glucose insulinotropic peptide (GIP) modulate post-prandial lipoproteins through a multitude of pathways that are independent of insulin and weight loss. Evidence from both animal models and humans seems to suggest an important effect on triglyceride rich lipoproteins (Apo48 containing) with little to no effects on other lipoproteins at least in humans. Dipeptidyl peptidase-4 (DPP4) inhibitors also appear to share these effects suggesting an important role for incretins in these effects. In this review, we will summarize lipid modulating effects of incretin analogs and DPP-4 inhibitors in both animal models and human studies and provide an overview of mechanisms responsible for these effects.

Keywords

apoB-48; DPP-4; GLP-1; incretins; lipoproteins

Background

The increasing prevalence of Type 2 diabetes mellitus (T2DM) has become a worldwide health concern [1]. While there are many underlying factors that predispose a diabetic patient to increased cardiovascular risk, lipoprotein abnormalities are frequent and are an important cause for cardiovascular events. The dyslipidemia of T2DM commonly manifests as elevation of both fasting and postprandial triglyceride-rich lipoproteins (TRL: VLDL,

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IDL and chylomicrons), decreased high-density lipoprotein cholesterol and increased small, dense LDL [2]. A fundamental issue associated with diabetic dyslipidemia is the overproduction of apoB-48 and apoB-100 lipoproteins and abnormal clearance capacity (lipolysis) which represents a functional consequence of insulin resistance associated with this condition [3]. Nonfasting plasma triglyceride (TG) concentration is a strong predictor of cardiovascular disease risk, independent of traditional cardiovascular disease risk factors [4,5]. Although the precise mechanisms by which triglycerides modulate cardiovascular risk is currently evolving, one hypothesis is that the increased risk associated with TRL may be due to the atherogenic and inflammatory properties of these particles generated in the postprandial state. Remnant particles of chylomicrons following hydrolysis are small enough for entry into the subendothelial space of the vascular wall and are postulated to contribute to atherosclerosis. Given the fact that humans spend a considerable amount of time in a postprandial state, there has been resurgent interest in modulating postprandial lipid metabolism in T2DM. The recognition of a gut-based peptide system for regulation of glucose has also led to the appreciation that, regulation of postprandial lipoprotein metabolism may also represent an important additional function of gut-derived peptides and indeed could represent a target for therapy.

Postprandial dyslipidemia as a risk factor

A substantial period of the day is spent in the postprandial and postabsorptive periods with the fasting period appearing as an anachronic period of daytime [6]. Fasting levels of glucose and lipids although useful from a diagnostic test standpoint may not reflect the postprandial state. In T2DM, the postprandial phase is longer and associated with greater and much more prolonged elevation of postprandial glucose and lipoproteins. Zilversmit postulated a role for postprandial lipoproteins, an association that has been validated in recent studies [7,8]. High concentration of remnant lipoproteins correlate with inflammation and are associated with increased risk of ischemic heart disease [9]. In the Copenhagen study, increased levels of remnant lipoproteins were associated with cardiovascular events [9]. Currently, although therapies such as fibrates are valuable adjuncts to statins in treatment of residual risk, the absolute benefit with these agents is modest and statin-fibrate combinations increased the risk of rhabdomyolysis. In addition, these agents appear to be effective only in selected subsets of individuals (i.e., fasting TG >205 mg/dl) [10]. In healthy individuals, insulin secretion increases in the postprandial phase, leading to a reduction in the secretion of endogenous lipoprotein synthesis (VLDL) by the liver. This allows normal handling of the intestinally derived lipoproteins which are synthesized following a meal, which share a common catabolic pathway through lipoprotein lipase with endogenously derived lipoproteins. In T2DM, even in well-controlled individuals with normal fasting TG levels, there is a substantial increase in the endogenous VLDL fraction during the postprandial phase, as indicated by the significantly higher apoB-100 in large VLDL (Sf 60–400 fraction) as well as an increase in apoB-48 levels [11,12]. The increase in apoB-48 reflects intestinally derived lipoproteins and reflects the exogenous pathway. Fasting serum apoB-48 is strongly correlated with postprandial lipemia and has been proposed as a marker of postprandial lipemia [13] and is indeed elevated in T2DM [14,15]. These particles have been shown to be associated with impairment of endothelial function

and asymptomatic peripheral arterial disease in patients with T2DM [16,17], and correlate with coronary artery disease and carotid intima-media thickness in subjects with normal serum TG levels [18,19]. The potential mechanisms by which lipoproteins such as apoB-48 play a role in the atherosclerotic process, besides mechanisms intrinsic to these particles such as inflammation may involve closely associated abnormalities in other lipoproteins such as HDL and LDL resulting in 'atherogenic lipoprotein phenotype' (low HDL and small dense LDL) [20,21].

Incretin hormones & control of lipids

The discovery of gut-derived hormones was initially meant to provide mechanisms for control of the postprandial state, with enhanced secretion of gut peptides reducing gut motility, decreasing appetite and facilitating nutrient disposal. However, the use of receptor antagonists and knockout mice has revealed that some gut hormones also exert physiologically important actions when present at low levels in the interprandial and fasting states. For example, infusion of glucagon-like peptide-1 (GLP-1) receptor (GLP-1R) antagonists increases fasting glucagon and glucose levels in multiple species, and mice genetically modified to lack the *GLP-1 receptor* (*Glp1r*^{-/-}) exhibit increased levels of fasting glucose [22–24]. Conversely, activation of GLP-1R signaling in the fasted-overnight state reduces glucagon and decreases fasting glucose levels in rodents and humans, contributing an important component of its therapeutic glucose-lowering effect [24]. These findings provide a rationale for the pharmacological activation of gut hormone pathways during both the postprandial and fasting states to optimize control of metabolic factors. Glucose-dependent insulintropic polypeptide (GIP) was the first incretin hormone identified and is secreted from enteroendocrine K cells in the proximal jejunum [25,26]. GLP-1 is the second incretin to be characterized as secreted as GLP-1(7–36) or GLP-1(7–37) and collectively referred to as intact bioactive GLP-1 [27]. GLP-1 is synthesized and secreted from enteroendocrine L cells present throughout the small and large intestines [24,26]. GLP-1 is also produced in the CNS, primarily in the brain stem, and transported to multiple regions within the CNS and exerts its anorectic effects via GLP-1 receptor activation [28–30]. GLP-1 is secreted from the intestine at low basal levels in the fasted state with circulating GLP-1 levels increase almost equal to two to threefold after a meal. Although the primary biological actions ascribed to intact GLP-1 are mediated by a single GLP-1R, GLP-1 is rapidly cleaved by dipeptidyl peptidase-4 (DPP-4) into GLP-1(9–36), both intact GLP-1 and GLP-1(9–36) can be further cleaved by neutral endopeptidase (Figure 1). Although it was widely believed that GLP-1 and GIP levels are reduced in T2DM, recent studies seem to indicate postreceptor defects in GLP-1 and GIP signaling [26].

Control of lipoproteins by incretin hormones

The idea of incretin-mediated changes in intestinal lipid metabolism was first demonstrated in rats by Qin *et al.* when effects of recombinant GLP-1 infusion were studied in intestinal lymph duct-cannulated rats, simultaneously infused with lipids through a duodenal cannula. Infusion of rGLP-1 at 20 pmol/kg/min caused a dramatic and prompt decrease in lymph flow and was accompanied by a reduction in apoB and apoAIV synthesis and triolein absorption [31]. These experiments were confirmed in humans, by Meier *et al.* who

demonstrated an effect of GLP-1 in regulation of postprandial TG and nonesterified fatty acid (NEFA) levels. GLP-1 administration lowered fasting and postprandial glycemia with delay in gastric emptying when compared with placebo. After meal ingestion, TG plasma levels increased in the placebo, an effect, completely abolished by GLP-1 (change in triglycerides, -0.023 ± 0.045 mmol/l; $p < 0.05$). During GLP-1 infusion, plasma concentrations of NEFA were suppressed by 39% in the fasting state and by 32% after meal ingestion ($p < 0.01$ for both) [32]. These results clearly demonstrated an effect of lipoproteins that may relate to multiple pathways including effects on gastric motility. Studies on DPP-4 inhibition, however, would seem to suggest that the effects of these agents may be unrelated to gastric emptying or related to weight loss and consequent improvement in insulin resistance. In a double-blind crossover study of vildagliptin (50 mg b.i.d.) versus placebo in T2DM for 10 days, gastric emptying, meal appearance and glucose kinetics were studied simultaneously. Gastric emptying, meal appearance postprandial glucose, insulin and C-peptide were not different between the two groups. Thus reduction in postprandial glucose control and perhaps lipids does not appear to depend on gastric emptying or the rate of entry of ingested glucose into the systemic circulation in humans [33].

In experiments performed on mice deficient for GLP-1R and hamsters treated by GLP-1 antagonist exendin 9–39, Hsieh *et al.* provided direct evidence that GLP-1R modulates postprandial lipid level [34]. GLP-1 agonist exendin-4 decreased TG and cholesterol in VLDL/chylomicron remnant sized lipoproteins. The levels of apoB-48 were reduced 90 min following fat load with exendin-4. The GLP-1R antagonist exendin 9–39 blocked the effects of exendin-4 on apoB-48. Consistent with data obtained with exendin 9–39 in hamsters, TG accumulation in lipoproteins as well as apoB-48 levels were increased in *Glp1r*^{-/-} subjected to oral olive oil gavage and Triton WR-39 infusion, despite the fact that *Glp1r*^{-/-} mice had gastric emptying that was comparable to wild-type mice. These experiments also confirmed the lack of dependence of lipid-lowering effects with GLP-1 on reduction of gastric motility. A nondegradable analog of GIP-1D-Ala2-GIP significantly increased plasma TG and apoB-48 following a fat load in hamsters demonstrating that another physiologic incretin GIP had opposing effects to GLP-1 on lipoproteins. Lee *et al.* documented direct effects of GLP-1 on the liver using an adenoviral overexpression approach of GLP-1 in ob/ob mice. GLP-1 overexpression in ob/ob mice markedly improved insulin resistance including increased hepatic expression of IRS-1 and activation of IRS-1 concomitant with a reduction of hepatic gluconeogenesis through reduced expression of enzymes such as phosphoenolpyruvate carboxykinase, glucose-6-phosphatase [35]. In the same study, the authors confirmed expression of GLP-1 receptor in human liver tissue and HepG2 cells. They demonstrated reduction of GLP-1 receptor in patients with nonalcoholic steatohepatitis. Trevaskis *et al.* examined the effects of the GLP-1R agonist AC3174 in a model of nonalcoholic steatohepatitis induced by feeding high-transfat, high-fructose and high-cholesterol diet.

Svegliati-Baroni evaluated the expression of GLP-1R. GLP-1R was expressed in both human liver biopsies and in high-fat fed diet (58% of energy derived from fat) Sprague-dawley rats [36]. The expression of GLP-1R decreased with high-fat diet. *In vitro* incubation of cultured hepatocytes derived from high-fat fed rats with Exenatide (1–100 nm) dose

independently upregulated the expression of PPAR- γ , PPAR- α , CPT1a and ACOX1. Further, Exenatide reduced abnormal JNK-1 and 2 phosphorylation, an effect that was countered by inhibition of PPAR- γ . Finally, Parlevliet *et al.* tested the effects of CNTO3649 and exendin-4 on *apoE*Leiden* Transgenic mice. Both agents reduced VLDL-TG and cholesterol individually and proportionately, suggesting an impact on VLDL particle production. This occurred in association with a reduction in hepatic content of TG and downregulation of genes involved in hepatic lipogenesis (*SREBP-1c*, *FASN*, *DGAT1*) [37].

In contrast to these positive studies, Panjwani *et al.* evaluated the effects of a long-acting GLP-1 receptor agonist Taspoglutide in *apoE^{-/-}* mice rendered diabetic using streptozotocin to induce modest hypoglycemia. Taspoglutide administration significantly reduced food intake and body weight. Hepatic TG and cholesterol levels were markedly reduced together with morphologic improvement (reduction in weight and reduced nodularity) and increase in liver X receptor as well as multiple liver X receptor target genes including *ABCA1*, *ABCG5*, *ABCG1* and *ABCG8*. Hepatic lipase as well as CPT1A, a rate-limiting mitochondrial enzyme controlling β -oxidation were also increased [38]. The authors further tested the expression of GLP-1 receptor, in additional acute experiments performed in C57BL/6 mice fed high-fat chow for 4 weeks, a single exendin-4 infusion delivered peripherally (10 or 24 nmol/kg) or intra-cerebroventricularly (0.023 and 0.23 nmol/kg) reduced peripheral TG. Given the fact that central infusion of a single dose of GLP-1 induced nearly identical effects on TG levels as peripheral administration of GLP-1, the authors posited that the effects of GLP-1 may be indirect, rather than through the direct activation of GLP-1R in the liver. Consistent with this hypothesis, GLP-1R could not be detected to an appreciable and consistent extent in murine liver tissue or cells [38]. The references reporting the lipid-lowering effect of GLP-1 agonists or DPP-4 inhibitors are summarized in Table 1.

Is the effect of GLP-1 on lipoproteins related to weight loss & anorectic effects of GLP-1?

The intuitive mechanisms by which pharmacologic doses of GLP-1 may improve postprandial lipids are through the effects on insulin secretion and improvement of insulin resistance. Intestinally derived apoB-48 lipoproteins and remnant particles may be cleared more effectively, perhaps concomitant with reduction of hepatically derived lipoproteins. Even in the absence of any effects on endogenous lipoprotein production, clearance pathways in the liver may be more effective for intestinally derived lipoproteins as both compete for similar pathways for clearance. Although improvement in insulin resistance as a mechanism to reduce postprandial lipidemia in T2DM has been suggested, GLP-1 may have often been observed to induce acute effects of lipoprotein metabolism without obvious changes in plasma insulin. Additionally, GLP-1 plays a far more complex role independent of insulin in regulating lipoprotein metabolism. For instance, GLP-1 exerts direct effects on the liver and CNS action and triggers effects that may be independent of insulin secretion. To what extent weight loss and appetite-independent effects of GLP-1 analogs contribute to improvement in insulin resistance and secondary reductions in postprandial lipoproteins has been investigated through acute studies involving single dose of a GLP-1 analog. Shirazi *et al.* demonstrated that activation of CNS GLP-1R by central injection of exendin-4 induced

anorexia and body weight loss through inflammatory pathways. Pharmacologic disruption of CNS IL-1 receptor or IL-6 biological activity attenuated anorexia and body weight loss induced by central exendin-4 administration in a rat [39]. In a double-blinded, randomized, placebo-controlled, crossover study in 35 subjects with impaired glucose tolerance or T2DM, a single subcutaneous injection of exenatide (10 µg) or normal saline was administered just prior to a high-calorie, fat-enriched breakfast meal with plasma lipoprotein measurements being performed up to 8 h post administration. Exenatide markedly reduced postprandial elevation of TG, apolipoproteins B-48, RLP-cholesterol and RLP-TG (all $p < 0.001$). Postprandial declines in NEFA were less pronounced but persisted longer with exenatide compared with placebo. Postprandial elevation of apoCIII was significantly attenuated by Exenatide [40]. Xiao *et al.* performed a detailed randomized crossover trial in 15 nondiabetic men who underwent two studies each (10 µg sc. exenatide vs placebo), 4–6 weeks apart, in which TG lipoprotein kinetics were examined with a primed, constant infusion of deuterated leucine and analyzed by multicompartmental modeling under pancreatic clamp conditions [41]. A fed state was maintained during each study by infusing a high-fat, mixed macronutrient, liquid formula at a constant rate directly into the duodenum via a nasoduodenal tube to tease out effects unrelated to gastric motility. Exenatide significantly suppressed the plasma concentration and production rate of apoB-48, but not of TG-rich apoB-100. The effects on apoB-48 were related to suppression of production rather than enhanced catabolism of particles. In the study, despite clamp conditions with constant infusion of insulin, glucagon, growth hormone and somatostatin, circulating insulin and C-peptide concentration increased immediately after exenatide injection, indicating exenatide-mediated enhancement of glucose-mediated insulin secretion. A recent study showed obese patients (both diabetic and normoglycemic obese patients) had increased brain responses to food pictures in appetite- and reward-related brain regions (insula and amygdala) and GLP-1 agonism by exenatide reduced food-related brain responses and appetite in obese patients, suggesting GLP-1 might be involved in obesity and obesity-related metabolic dysregulation [42].

DPP-4 inhibition & effects on lipoprotein metabolism

Given the effects of GLP-1 at pharmacologic levels in reducing postprandial lipids, it has been unclear if inhibition of GLP-1 degradation through enzymatic inhibition of DPP-4 would lower postprandial levels, and if so, to a degree similar as exogenous administration of a GLP-1 agonist. In a 4-week single center, randomized double-blind study of 31 drug-naïve individuals with T2DM, Vildagliptin (50 mg b.i.d.) was compared with placebo. At baseline and following 4 weeks, patients underwent a standard fat-rich test meal with measurement of lipids subfraction(s) over 8 h following the test meal. Vildagliptin had no effect on fasting total TG, total cholesterol or serum apoB relative to placebo but decreased the area under the curve following the test meal for total plasma TG when expressed as the percentage change from baseline by $22 \pm 11\%$ ($p = 0.04$). This was associated with a decrease in chylomicron apoB-48 and cholesterol but no changes in apoB-100 in either the chylomicron or the VLDL/IDL subfractions. Vildagliptin also increased intact GLP-1, suppressed inappropriate glucagon secretion and decreased fasting as well as postprandial glucose [43]. The effects of DPP-4 inhibition appear to be a class effect as this has been

demonstrated with other analogs. In a randomized crossover study with alogliptin by Noda *et al.* involving healthy adults, treatment with alogliptin at 25 mg per day for a week resulted in suppression of postprandial elevation of triglycerides, apoB-48 and RLP-cholesterol. Interestingly, postprandial endothelial dysfunction was associated with change in postprandial RLP-cholesterol [44]. Tremblay *et al.* randomized 36 subjects with T2DM in a double-blind crossover study using sitagliptin 100 mg per day or placebo for a 6-week period each. At the end of each phase of treatment, patients underwent an oral lipid tolerance test. Sitagliptin therapy significantly decreased the postprandial area under the curves for plasma apoB-48, TG, VLDL-cholesterol, free fatty acids and glucose. Sitagliptin therapy also improved homeostasis model assessment HOMA-IR and β indices [45]. In a recent paper by Xiao *et al.*, TRL kinetics in response to a single dose of sitagliptin in healthy subjects was tested following a high-fat, liquid formula every hour to maintain a constant fed state, under pancreatic clamp conditions [46]. Sitagliptin decreased TRL apoB-48 concentration by reducing production rates as compared with placebo. Pancreatic hormones, FFAs and glucose levels were similar between placebo and sitagliptin. This effect on reduction of intestinal lipoprotein production was similar to the GLP-1R agonist exenatide shown in a previous by Xiao *et al.* [41]. Consistent with the effects of exenatide, sitagliptin did not significantly affect the plasma concentration or production of hepatic lipoproteins although it decreased lipoprotein particles of intestinal origin [46]. These findings suggest that the lipid-lowering effects of DPP-4 inhibitors may relate to their effects on GLP-1. In addition to the beneficial effects of incretin therapy on postprandial lowering of lipids, the insulotropic effects and restoration of insulin sensitivity may indeed reduce fasting triglycerides. Together with weight loss that occurs when used chronically, a reduction in fasting lipid levels may occur. This has been seen in animal studies that demonstrate reduction in fasting triglycerides. In humans, multiple studies have demonstrated reductions in fasting triglycerides with single doses suggesting that these effects on fasting lipids are unrelated to weight loss [32,40]. Similar findings have also been demonstrated in the chronic administration of the drug. In an integrated analysis of six DURATION trials conducted by Grimm *et al.*, examining the effects of a once weekly Exenatide preparation in more than 1300 patients, for 24–30 weeks, reduction in the total cholesterol (–6.5 mg/dl [–8.2 to –4.7 mg/dl]), LDL-cholesterol (–3.9 mg/dl [5.3 to –2.5 mg/dl]) and triglyceride levels (–6% [–8 to –4%]).

Additional mechanisms by which GLP-1 & DPP-4 inhibition improves postprandial lipids

Another mechanism may relate to reduction in apoCIII. ApoCIII is a small protein that resides on the surface of VLDLs, low-density lipoproteins, chylomicrons and high-density lipoproteins. ApoCIII is a major regulator of lipolysis, as it noncompetitively inhibits endothelial-bound lipoprotein lipase, the enzyme that hydrolyzes triacylglycerols in TG-rich lipoproteins, which generates smaller remnant lipoproteins. It also inhibits the uptake of lipoprotein remnants by hepatic lipoprotein receptors, while high concentrations of apoCIII inhibit hepatic lipase, an enzyme, further reducing lipolysis [47]. In a double-blinded, randomized, placebo-controlled, crossover study, Schwartz *et al.* demonstrated that a single subcutaneous injection of 10 μ g Exenatide prior to a high-calorie, fat-enriched breakfast

meal reduced postprandial elevation of apoCIII and B-48, along with RLP-cholesterol and triglycerides [40]. Considering the importance of apoCIII in postprandial lipid metabolism, the decrease in apoCIII may represent an additional mechanism by which GLP-1 analogs may decrease postprandial lipids.

In contrast, levels of GLP-1 with DPP-4 inhibition are much lower compared with pharmacologic treatment with GLP-1 analogs and since increase in circulating GLP-1 and insulin levels postprandially is not consistently observed, to what extent does DPP-4 inhibition result in reduction in postprandial lipoproteins through GLP-1 mediated effects (including those on gastric motility) is a relevant question. For instance in a crossover study with vildagliptin performed over 6 weeks by Azuma *et al.*, vildagliptin increased postprandial GLP-1 and GIP by three and twofold, respectively, reduced fasting and postprandial plasma glucose and improved glucose responsiveness of insulin secretion by 50%. Examined by glucose clamp, insulin sensitivity and glucose clearance improved after vildagliptin [48]. In order to further address direct effects of DPP-4 inhibition on adipose and skeletal muscle metabolism including enhanced lipolysis in target tissues, Boschmann *et al.* randomized 20 patients to Vildagliptin or placebo in a randomized crossover study over 7 days. Microdialysis catheters were placed in the adipose tissue in the abdomen and into the quadriceps femoris (vastus lateralis) muscle. Microdialysate perfusate (inflow) and dialysate (outflow) ethanol concentrations were assayed using the ethanol dilution technique. In the latter technique, ethanol is added to the perfusate and diffuses into the interstitial space where it is removed via capillaries. The higher the capillary blood flow, the higher the removal of ethanol out of the tissue with the ratio of ethanol concentration in the dialysate versus perfusate representing a semiquantitative measure of blood flow in the tissue. Adipose tissue and skeletal muscle blood flow were similar with placebo and with vildagliptin, both before and during ingestion of the test meal. However, glycerol concentrations were increased in adipose tissue with vildagliptin suggestive of increased adipose lipolysis. Interestingly, there was a marked reduction in postprandial lactate and pyruvate production in the skeletal muscle with DPP-4 inhibition. These effects may reflect improvement in hepatic glucose production by the liver due to obviation of insulin resistance and enhanced peripheral lipolysis. These results were supported by indirect calorimetry results that seemed to suggest increased energy expenditure and enhanced lipid oxidation rates [49].

GLP-2 role in lipoprotein metabolism & effects of combined GLP-1 & GLP-2

GLP-2 is co-secreted with GLP-1 in a 1:1 molar ratio from the intestinal L cells and plays opposing effects on lipoprotein metabolism through its effects on the GLP-2 receptor. The latter is a member of the family B secretin-like subfamily of G-protein-coupled receptors, is expressed in stomach, small and large bowel, brain and lung. The intestine is the principal target tissue of GLP-2 and the highest expression of GLP-2 receptors is in the enteroendocrine cells in the proximal intestine (jejunum) [50]. Although both GLP-1 and GLP-2 are both substrates of DPP-4, GLP-2 is less rapidly degraded resulting in sustained elevations in GLP-2. GLP-2 plays a significant role in regulation of bowel mass and mucosal integrity, stimulation of enterocyte proliferation and prevention of apoptosis. The effects of GLP-2 effects on lipoproteins are consistent with its effects in stimulating uptake

of luminal carbohydrates, amino acids, hexose transport and increasing nutrient transport genes [50]. In hamsters, GLP-2 stimulates intestinal apoB-48-containing lipoprotein secretion, possibly through increased absorption of luminal lipid [51]. In healthy humans, intravenous infusion of GLP-2 for 390 min led to higher postprandial plasma concentrations of FFA and TG, without inhibiting gastric emptying or stimulating insulin secretion [52]. With the opposing effects of GLP-1 and 2, what are their relative contributions to lipoprotein metabolism? In a study in hamsters, co-infusion of GLP-1 and GLP-2 (1:1) for 30 min elevated TRL TG and apoB-48. In contrast, with more prolonged co-infusion (120 min) or treatment with sitagliptin, postprandial lipemia was reduced [53]. It appears that, under normal conditions, GLP-2 action predominates while sustained GLP-1 action (e.g., via DPP-4 inhibition) converts this predominance to attenuate postprandial lipemia. Insulin resistance as a result of fructose feeding is associated with enhanced GLP-2 action and diminished GLP-1 action and thus may represent an opportunity to interrupt GLP-2. In a recent study, the therapeutic efficacy of a peptide with balanced co-agonism at both GLP-1 and GIP was tested. The duration of action of the dual incretins was enhanced through site-specific PEGylation to support less frequent administration. The unimolecular dual incretin demonstrated enhanced antihyperglycemic and insulinotropic efficacy relative to GLP-1 agonists across multiple models of obesity and diabetes, including db/db mice and ZDF rats, cynomolgus monkeys and humans [54]. The unimolecular dual incretins corrected insulin resistance and insulin deficiency simultaneously and more effectively than mono-agonists. This discovery and validation of a balanced and high-potency dual incretin agonist suggests a new paradigm for the management of diseases associated with impaired glucose tolerance. However, the clinical efficacy of these agents will need testing in large appropriately designed clinical trials.

Conclusion

Endogenous incretins such as GLP-1 and GIP clearly have important effects on lipoprotein pathways (Figure 2). Pharmacologic doses of GLP-1 clearly reduce postprandial lipids and lower apoB-48 production and TG through gastric motility independent effects. The effects on endogenous lipoproteins and hepatic pathways are of a little uncertain, given the current controversy regarding expression of GLP-1 in liver to a substantial degree. DPP-4 inhibition often in the short and in the absence of weight loss may lower postprandial lipids. The demonstration of effects on fasting lipids is less impressive and indeed the demonstration of an effect of these agents may depend on carefully designed *a priori* protocols to investigate postprandial lipid metabolism. Modulation of postprandial lipid metabolism may represent a reasonable mechanism for lowered risk with incretin analogs and DPP-4 inhibitors in clinical trials of these agents.

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Executive summary

Postprandial lipid dysregulation in Type 2 diabetes mellitus

- Greater and prolonged elevation of postprandial lipoproteins in Type 2 diabetes mellitus patient is associated with longer postprandial phase.
- Increased level of postprandial remnant lipoprotein in Type 2 diabetes mellitus correlates with inflammation and is associated with increased risk of ischemic heart disease.

Incretin effects on lipid metabolism

- GLP-1, signaling through GLP-1R, reduced remnant lipoprotein and CM production.
- DPP-4 inhibition increased plasma GLP-1 level and reduced plasma triglyceride, very-low-density lipoproteins and CM remnants.

Future perspective

- GLP-1 and DPP-4 inhibitors may be used preferentially in patients with marked abnormalities in postprandial lipid metabolism;
- The use of GLP-1 in combination of GLP-2 in the form of novel incretins ('twincretin') may be more effective than either agent alone.

Figure 1. Production and degradation of incretin hormones

GLP-1 is produced by L cells predominantly located in distal gut, while GIP is produced by K-cells in proximal gut. Both GLP-1 and GIP are rapidly degraded by DPP-4.

DPP-4: Dipeptidyl peptidase-4; GIP: Glucose-dependent insulintropic polypeptide; GLP-1: Glucagon-like peptide-1.

Figure 2. GLP-1 mediated postprandial lipid regulation

GLP-1 regulates postprandial lipemia via mechanisms in multiple organs involved in control of lipid metabolism including the liver, pancreas, intestines and adipose tissue. GLP-1 is produced in the gut in response to a meal. By acting on pancreatic β cells, GLP-1 promotes insulin secretion which subsequently modulates postprandial blood glucose level and reduces plasma FFAs through inhibition of lipolysis in adipose tissue. GLP-1 can also reduce VLDL production through direct effects on the liver and via suppression of apoB-48 and CM biogenesis in the gut via both direct and indirect effects such as reducing lymph flow, suppressing gastric emptying and inhibiting gastric lipase. CM: Chylomicron; FFA: Free fatty acid; TG: Triglycerides.

Table 1

Published literature on the effect of GLP-1 agonists and DPP-4 inhibitors on lipid metabolism.

Treatment	Dose	Subject	Changes in lipid metabolism	Ref.
rGLP-1	20 pmol/kg/min infusion	Rat	rGLP-1 treatment reduced lymphatic apoB and apoA-IV; cholesterol absorption: no change	[31]
GLP-1	1.2 pmol/kg/min infusion	Human	TG: -0.023 ± 0.045 mmol/l	[32]
Vildagliptin	50 mg b.i.d.	Human	Vildagliptin decreased the area under the curve of plasma TG following the test meal by $22 \pm 11\%$. This was associated with a decrease in chylomicron apoB-48 and cholesterol	[43]
Vildagliptin	100 mg/day for 7 days	Human	Vildagliptin increased glycerol and lactate concentrations in adipose tissue suggestive of increased adipose lipolysis	[49]
Sitagliptin	5 mg/kg/day in hamster for 3 weeks	Hamster	Sitagliptin reduced VLDL-TG (by threefold), TRL-TG, TRL-cholesterol and TRL-apoB-48	[34]
Exendin-4	24 nmol/kg in mice and 5 nmol/kg in hamsters	Mouse and hamster	Exendin-4 decreased TG and cholesterol in VLDL/chylomicron remnant sized lipoproteins. Exendin-4 also decreased apoB-48 level (Exendin-4 71.61 ± 17.41 vs Control 100.00 ± 10.24), an effect blocked by exendin 9–39	[34]
Exenatide	10 µg sc. single injection	Human	Exenatide markedly reduced postprandial elevation of TG, apoB-48, RLP-cholesterol and RLP-TG	[40]
GLP-1 analogs CNTO3649 or exendin-4	1.0 or 3.0 mg/kg/day (CNTO3649), or 15 or 50 µg/kg/day (exendin-4) sc. for 4 weeks	Mouse	The VLDL-TG production rate was reduced by CNTO3649 (up to -36% , $p < 0.01$) and exendin-4 (up to -54% , $p < 0.001$). Both CNTO3649 and exendin-4 reduced hepatic TG (-39% , $p < 0.05$ and -55% , $p < 0.01$, respectively)	[37]
Exenatide	10 µg sc.	Human	Exenatide suppressed the plasma concentration and production rate of apoB-100	[41]
GLP-1 agonist Taspoglutide	0.4 mg sc., once per month for 3 months	Mouse	Hepatic TG and cholesterol levels were markedly reduced by Taspoglutide	[38]
Exendin-4	10 or 24 nmol/kg infusion, or 0.023 and 0.23 nmol/kg icv.	Mouse	Both peripheral infusion and icv. injection of exendin-4 reduced peripheral TG	[38]
GLP-1; Sitagliptin	GLP-1 (20 pmol/kg/min infusion); Sitagliptin ip. (dose not specified)	Hamster	GLP-1 infusion decreased lipid absorption and levels of TRL-TG and apoB-48. In consistence, DPP-4 inhibition resulted in decreased postprandial lipemia (TRL-TG, TRL-cholesterol and intestinal CM production)	[53]
Alogliptin	25 mg/day for a week	Human	Alogliptin reduced postprandial TG, apoB-48 and RLP-cholesterol	[44]
Sitagliptin	100 mg/day for 6 weeks	Human	Sitagliptin decreased the postprandial area under the curves for plasma apoB-48, TG, VLDL cholesterol and free fatty acids	[45]
Sitagliptin	100 mg oral	Human	Sitagliptin decreased intestinal lipoprotein concentration by inhibiting particle production	[46]

b.i.d.: bis in die; DPP-4: Dipeptidyl peptidase-4; GLP-1: Glucagon-like peptide-1; icv.: Intracerebroventricular injection; ip.: intraperitoneal injection; RLP: Remnant-like particle; sc.: Subcutaneous injection; TG: Triglyceride; TRL: Triglyceride-rich lipoprotein.

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